



Comparison of Different Doses of Petroleum Ether Extracts of *Sphaeranthus indicus* Linn Against Dutasteride on Testosterone and PSA in Murine Model of Benign Prostatic Hyperplasia

¹Sabiha Erum Khan, ²Bisma Fatima Aslam, ¹Saadia Shahzad Alam

¹Department of Pharmacology, Shaikh Zayed Medical Complex, Lahore

²Department of Pharmacology, AzraNaheed Medical College, Lahore

ABSTRACT

Introduction: Benign Prostatic Hyperplasia (BPH) is the most common health problem of male elderly population resulting in lower urinary tract symptoms (LUTS), characterized by frequency, urgency, hesitancy, nocturia, dysuria and incomplete voiding. **Aims & Objectives:** To compare the different doses of petroleum ether extracts of *Sphaeranthus indicus* Linn (*SiP*) against *Dutasteride*, on hormonal parameters (serum testosterone and PSA) in testosterone induced BPH in albino mice. **Place and Duration of study:** This experimental study was conducted at the Animal House of PGMI, Lahore for 6 months. **Material & Methods:** Thirty-six healthy adult male mice, divided into 6 groups, were administered testosterone, different doses of petroleum ether extract *SiL* extracts (*SIP*) and dutasteride. Animals in group 1, taken as control, were given 0.1ml corn oil daily subcutaneously for 28 days. The animals in the remaining 5 groups (# 2-6) were given 3 mg/kg body weight testosterone dissolved in corn oil subcutaneously for 28 days, to induce BPH. The animals in group 3 were given 20 mg/kg/day Dutasteride orally for 28 days. Animals in groups 4-6 were given 25,50mg and 75mg/ kg body weight petroleum ether extract (*SIP*) orally for 28 days. Blood samples were drawn on day 0, 14 and 28 for the estimation of serum testosterone and serum PSA, by ELISA technique. Data was analyzed using SPSS Version 20.0. Comparison (between groups at baseline, day14 and day 28) was done by using one-way ANOVA and Post Hoc Tukey's test **Results:** Decrease in PSA level and increase in serum testosterone supports the alpha reductase inhibiting activity of *SiP*, in a dose dependent manner, in testosterone induced BPH. *SiP* petroleum ether extract 75mg/kg exhibited efficacy similar to that of dutasteride. **Conclusion:** *Sphaeranthus indicus* Linn (*SiP*) has shown efficacy equal to that of dutasteride in attenuating the testosterone induced BPH, in albino mice.

Key words: Benign prostatic hyperplasia, testosterone, prostate specific antigen, Dutasteride, *Sphaeranthus indicus* Linn (*SIL*), Petroleum ether extract of *Sphaeranthus indicus* Linn (*SiP*).

INTRODUCTION

“I do not need you to remind me of my age.

I have a bladder to do that for me”.

(Stephen Fry—author of “The Fry Chronicles”)

Benign prostatic hyperplasia (BPH) is the most common, although benign, bothersome malady causing significant urinary symptoms in the male elderly population.¹ Based on histology, the term refers to proliferation of smooth muscles and epithelial cells situated in the transition zone of the gland.²

BPH could progress unchecked, if not managed rationally, leading to Lower Urinary Tract

Symptoms (LUTS) manifested by weak urinary stream, hesitancy, incomplete voiding and irritative symptoms including frequency, urgency, nocturia and dysuria.³ Incomplete voiding results in stasis of residual urine with an increased risk of recurrent urinary infections. Chronic urinary retention may result in renal failure in some patients.⁴ Generally, it is not a life-threatening condition.⁵ However, it is well known to have significant detrimental effect on a patient's quality of life (QoL).⁶

Whereas the incidence is on increase due to an increase in average life span, the threat of BPH is hanging over most of elderly men as the “Sword of Damocles”.⁷ It affects 50 percent men between 51 and 60 years and about 80 percent in their eighties.⁸

The etiology is not clearly understood.⁹ However, the development of BPH is associated with the presence of testicular androgens during prostate development and growing age.¹⁰ The major portion of Testosterone, in men, is produced by Leydig cells of the testes but also, to a lesser extent, by adrenal cortex. In the body tissue, the testosterone is converted into Dihydrotestosterone (DHT) which is more potent and plays an important role in the development of prostate. However, the presence of DHT, in adulthood, could be injurious as it would result in unchecked growth of the gland.¹¹ The physiologically well characterized 5AR substrate, the androgen testosterone and its more potent metabolite DHT are essential hormones responsible for male phenotypic sexual differentiation and maturation through their actions at the androgen receptor".¹²

PSA is chemically a serine protease, produced primarily by the glands in the transition zone and is androgen dependent. The highest amount of PSA is found in these seminal fluid; some amount of PSA escapes the prostate and could be detected in the serum. The rising levels of PSA in serum are associated with prostate cancer, however the levels may also rise in BPH. The normal value for total PSA in human blood is 4ng/mL. When between 4-10, it indicates possibility of BPH or cancer. The normal value of PSA levels in mice is 0.14-0.86ng/ml.¹⁴

Alpha blockers and 5- alpha reductase inhibitors are the two FDA approved treatment options. Administration of a 5- alpha reductase inhibitor (Dutasteride and Finasteride), offers rapid and sustained inhibition of DHT and is therefore the major treatment for BPH, at the present. The patients are at risk of acquiring drug related problems (DRPs), notably dizziness and orthostatic hypotension in case of alpha blockers and sexual dysfunction and gynecomastia by 5- alpha reductase inhibitors.¹⁵

Sphaeranthus indicus Linn (gorakmundi) which literally means round flower, in Greek, is a medicinal plant fascinating healthcare provider since centuries. It is an indigenous herb of South Asians being in use in Ayurvedic pharmacopeia, for many ailments. It grows frequently both in cultivated and un-cultivated lands, from sea level to 1200 m altitude, in Indian sub-continent, Malaysia, China, Africa and Australia.¹⁶ It is a spreading aromatic plant (becoming odorless on long standing) with spreading glandular stem and branches with purple or pink flowers.¹⁷

The phytochemical investigations of the plant revealed the presence of¹⁷:

- an alkaloid "*Sphaeranthine*"
- a novel "*Flavonoid C-glycoside*"
- *Stigmasterol and B-sitosterol*
- Bicyclic Sesquiterpene from petroleum extract

Although the efficacy of this medicinal plant in BPH was postulated earlier, the first published study, on its potential use in BPH, was conducted in India, in 2011, by Nahata and Vinod Kumar Dixit. They induced prostatic hyperplasia, in Albino rats, by giving them Testosterone. The administration of *SiL* was found to attenuate the action of Testosterone.¹⁸ To the best awareness of authors, no authentic published work is available on its potential use in BPH, in Pakistan.

Whereas Nahata and Dixit used Finasteride as positive control, we opted Dutasteride instead. It is already known that Finasteride (selective 5 α -reductase inhibitor) achieves 70% suppression which in case of Dutasteride (from non-selective group) reaches up to 94.8%.¹⁹

MATERIAL AND METHODS

Animals:

36 adult male mice of Balb C species, weighing 20-30 gm, were purchased and housed in University of Health Sciences, Lahore. They were kept in standard polypropylene cages, at controlled room temperature of 25 \pm 10°C and relative humidity 60-70%. Six groups of 6 animals each, were used for the experiment. Duration of study was total of 28 days.

Testosterone Propionate (Tevot 250 mg) injections were purchased from local pharmacy. All groups (except the control one) received 3mg/kg body weight Testosterone Propionate subcutaneously for 28 days, to induce BPH.²⁰

Dutasteride (Avodart 0.5 mg) tablets were purchased from the local pharmacy. Animals in group 3 were given 20 mg/kg/day Dutasteride orally for 28 days.

Mouse testosterone Elisa Kits and Mouse PSA Elisa kits were purchased from Glory Science Limited USA. Petroleum Ether was purchased from Merck Pharmaceuticals.

Plant Material:

Flower heads of *SiL* were collected from Lahore area. They were identified and authenticated from the herbarium maintained by the Department of Botany of University of the Punjab Lahore. They were kept at normal room temperature. Petroleum ether was prepared in the Chemistry Department of PCSIR Laboratory Lahore.

Preparation of Petroleum Ether Extract:

500gms of powdered flower heads of *SiL* were dipped in 2L petroleum ether, kept for 48 hrs with periodic shaking and left for half an hour to settle down. It was then filtered in a big flask using Whatman’s filter paper and glass funnel. The filtrate evaporated in rotary evaporator until all the petroleum ether was removed and dark greenish yellow waxy mass was obtained.¹⁸ From 500g herb, the extract obtained was 14gm (2.8% yield).

Animal Grouping:

Animals in group 1 (control group) were given 0.1ml corn oil daily subcutaneously for 28 days. The animals in the remaining 5 groups (2-5 groups) were given 3 mg/kg body weight of testosterone (containing 0.075 mg testosterone) dissolved in 0.1ml of corn oil subcutaneously for 28 days. Animals in group 3 were given 20 mg/kg/day Dutasteride orally for 28 days. Those in group 4, 5 and 6 received 25 mg, 50 mg and 75 mg/kg body weight *SiP* extract orally for 28 days.

Statistical analysis:

Data was analyzed by SPSS Version 20.0. The data for serum testosterone and serum PSA levels obtained by using mean±sd. Comparison (between groups at baseline, day14 and day 28) was done by using one-way ANOVA and Post Hoc Tukey’s Test. Lettering was assigned on the basis of Tukey’s Test. Different letters in superscript represented significant difference from each other and those having common letters were non significant with p-value 0.05. Cluster bar diagrams were made to give visual difference between groups at each reading time and error bars were used to show the standard deviations. P-value ≤ 0.05 was considered statistically significant.

RESULTS

The mean serum testosterone levels at baseline were 1.55 to 1.59 and had no significant difference (p-value 0.271). At day 14 the group 6 had highest level, significantly different from all other groups, followed by group 3, 5 and 4 respectively. The group 1 and 2 had no significant difference from each other on day 14 but had significantly lower mean testosterone levels as compared to all other groups. On day 28 the testosterone levels increased in all groups as compared to day 14 and group 3 had the highest mean level of 10.48 ng/ml, non significantly different from group 6 but significantly higher than all other groups. The group 2 also had significantly higher level as compared to group 1, but significantly lower than group 4 and 5. (Fig-1)

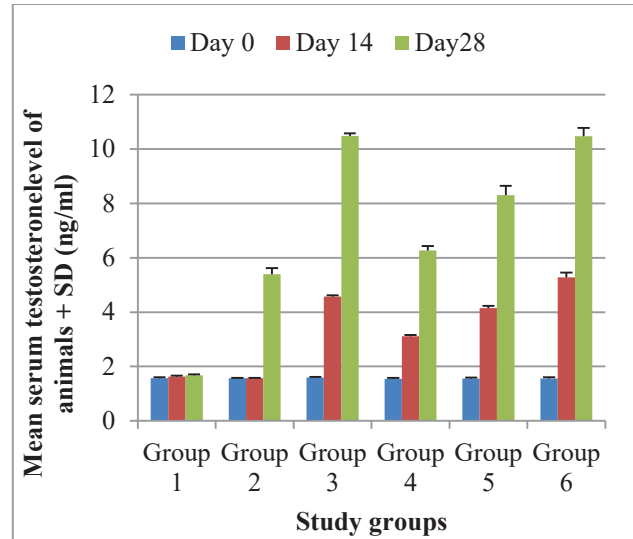


Fig-1: Cluster bar diagram presenting mean serum testosterone levels for six study groups (error bars presenting standard deviation)

The mean serum PSA levels, ranging between 0.22 and 0.25ng/ml at base line with standard deviations ranging between 0.02 and 0.04, were insignificantly different with p-value 0.651. On day 14 the mean levels in all groups (except group1) rose significantly, the highest being in group 2. Groups 3, 5 and 6 had no significant difference from each other, either on day 14 or day 28. Group 4 had significantly higher level in comparison to other groups but lower than group 2. The same was the difference on day 28, though the mean levels in all groups were higher as compared to day 14. (Table-2, Fig-2)

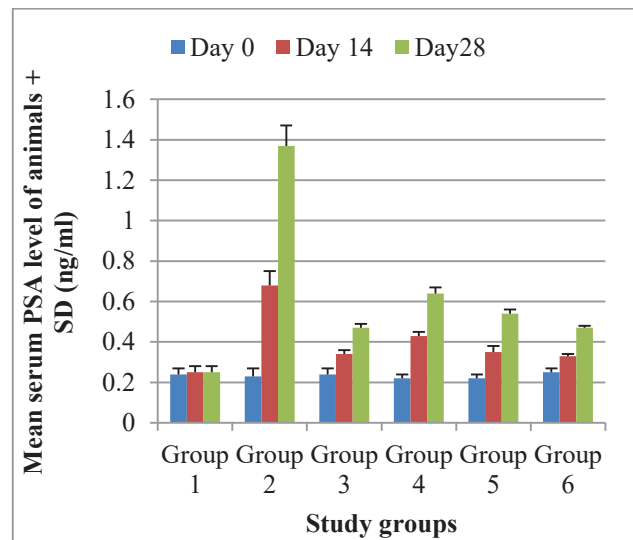


Fig-2: Cluster bar diagram presenting mean serum PSA levels for six study groups (error bars presenting standard deviation)

DISCUSSION

BPH is a progressive age-related health problem of elderly men. It is clinically characterized by prostatic enlargement and resultant LUTS causing significant detrimental effects on QoL of the life of the patients.^{1,6} It is not a premalignant condition and has anetiology different from prostatic cancer. A variety of growth factors and inflammatory process could contribute to the causation.⁹

Although many medical and surgical treatment options are available, the problems of the patients remain unresolved in a significant number of cases. The medical therapy does not work for everyone. The drug related problems have a serious impact. The surgical intervention may, in some cases, worsen the condition. The immediate post-operative complications include bacterial infection (up to 15%), impotence (upto 17%) and retrograde ejaculation (upto 77%). However, the incidence of actual post-operative complications, as concluded by Stefanie Petrou Binder, is higher than that reported in literature.²²

Phytotherapy, as a new treatment option for BPH, is gaining popularity on global level. *SiL* is in use in Ayurvedic System of traditional medicine, for many ailments.²³ It is an ingredient of "Prostabliss" which is said to have beneficial effects on BPH.¹⁴

The present study was conducted to assess the efficacy of various doses of petroleum ether extracts of this herb in comparison to dutasteride in testosterone induced prostatic hyperplasia in albino mice. Testosterone levels were measured in this study on day 0, 14 and 28 as a marker of 5 alpha reductase inhibition. All the groups (except the control one) showed an elevation of testosterone levels because of exogenous testosterone treatment. The effect on group 3 (Testosterone+Dutasteride) was quite similar to that in group 6 (Testosterone+*SiP*) manifesting an increment of 572.5% in serum testosterone level. Results show that Group 3 had significantly higher serum testosterone level than all the groups except 6 with a p-values <0.05. These findings support the alpha reductase inhibitory effect of *SiL*.²⁵

At day 28, the average mean serum PSA level of group 1 was 0.25ng/ml and that for group 2 was 1.37ng/ml. The comparatively highest level in group 2 (testosterone alone) could be explained because of the resultant prostatic hyperplasia. Group 1 had significantly lower levels as compared to all other groups while group 2 showed significantly higher PSA levels as compared to all other groups with p-values <0.05. It shows that the testosterone opposing effect of dutasteride was comparable with

SiP 50mg/kg and *SiP* 75mg/kg body weight. The only available comparable study (Nahata et al) also showed a decrease in serum PSA by beta sitosterol of *SiL*.¹⁸

A large multi-centered study is required to further evaluate the alpha reductase inhibitory effect of the fascinating herb. The results of the present study, if confirmed on a large scale, would, most hopefully, bring a revolutionary change in the management of BPH and the patients would be saved from the injurious untoward effects of the presently available therapeutic regime. This joint venture of the pharmacologists, urologists and pharmacists is the need of the day.

CONCLUSION

In conclusion, this fascinating herb has shown efficacy equal to that of dutasteride in attenuating the testosterone induced BPH in albino mice. The results suggest that petroleum ether extracts of *SiL* prevented prostatic hyperplasia significantly in a dose dependent manner. The best activity was exhibited by *SiP* 75 mg /kg body weight.

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The Authors:

Dr. Sabiha Erum Khan
P.G. Trainee,
Department of Pharmacology & Therapeutics,
Shaikh Zayed Medical Complex, Lahore.

Dr. Bisma Fatima Aslam
Demonstrator,
Department of Pharmacology & Therapeutics,
Azra Naheed Medical College, Lahore.

Prof. Saadia Shahzad Alam
HOD, Pharmacology & Therapeutics,
Shaikh Zayed Medical Complex, Lahore.

Corresponding Author:

Dr. Sabiha Erum Khan
P.G. Trainee,
Department of Pharmacology & Therapeutics,
Shaikh Zayed Medical Complex, Lahore.
E-mail: drsabihaerum@gmail.com